# Novel Dopamine Receptor Subtypes as Targets for Antipsychotic Drugs

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Dopamine (DA) is an important neurotransmitter involved in diverse cerebral functions, among which those that control hormone secretion, emotions, and motor and motivated behaviors. The molecular diversity of dopamine receptors, recently revealed by the approaches of molecular biology, indicates that DA may mediate its various functions by interacting with at least five different DA receptors encoded by different genes. These receptors can be classified in $D_1$-like and $D_2$-like subfamilies according to primary sequence homology, gene organization, pharmacology, and, to some extent, intracellular signaling.[1–4] Thus, the $D_1$-like receptors—$D_1$ and $D_5$ receptors—are encoded by intronless genes and coupled to $G_s$-mediated activation of adenylyl cyclase. $D_2$-like receptors—$D_2$, $D_3$, and $D_4$ receptors—are encoded by genes with their coding sequence interrupted by introns and mediate $G_i$ and $G_o$-mediated inhibitory responses, namely, inhibition of cAMP formation.[5–8]

Disturbances in DA neurotransmission have been implicated in several neuropsychiatric disorders, such as schizophrenia. Although it has been recognized that antipsychotic drugs primarily block DA receptors, it is unclear which precise target is involved. Moreover, the favorable therapeutic effect of currently used antipsychotic drugs is often impaired by severe motor and/or endocrine side effects, which limit their use. The possibility that blockade of the various DA receptors results in different clinical effects, for instance, favorable versus adverse effects, affords opportunities to select more efficient and safer drugs.

The DA receptor subtypes cannot be simply classified into two independent and functionally opposing receptor families, because they have been shown to participate in various reciprocal interactions. In spite of their opposite effects on cyclic AMP formation, $D_1$-like and $D_2$-like receptors display cooperative interactions that have been extensively illustrated in animal behavioral models.[9] More recently, functional communication at the molecular level between $D_1$ and $D_2$ receptors has been suggested,[10] possibly involving G-protein interactions.[11] Likewise, the $D_1$ receptor-induced facilitation of $D_2$ receptor-mediated arachidonate release[12] indicates that interacting intracellular signaling pathways may also account for synergism between

the receptors of the two subfamilies. On the other hand, $D_2$-like receptors cannot be regarded just as homologous and functionally related isoreceptors. We show here that $D_2$ and $D_3$ receptors, two likely targets for antipsychotic drugs, display distinct intracellular signaling pathways in a heterologous expression system and distinct modes of regulation in brain after interruptions of DA neurotransmission. Furthermore, $D_2$ and $D_3$ receptors blockaded by antipsychotic drugs have the opposite effects on neurotensin/neuromedin N expression in nucleus accumbens, a putative biochemical index of antipsychotic drug effects.[13–15] The therapeutical consequences of this dual interaction and differential regulation will be examined in the treatment of schizophrenia, a disorder characterized by the occurrence of both positive symptoms (hallucinations, delusions) and negative symptoms (impoverished thought and affect).

TABLE 1. Comparison of Dissociation Constants of Antipsychotic Drugs at Cloned Dopamine Receptor Subtypes[a]

| Antipsychotic Agent | $D_1$-like Receptors | | $D_2$-like Receptors | | |
|---|---|---|---|---|---|
| | $D_1$ Receptor | $D_5$ Receptor | $D_2$ Receptor | $D_3$ Receptor | $D_4$ Receptor |
| Haloperidol | 30 | 40 | 0.6 | 3 | 5 |
| Chlorpromazine | 16 | 33 | 2 | 6 | 37 |
| Thioproperazine | – | – | 0.5 | 1 | 50 |
| Thioridazine | – | – | 5 | 8 | 12 |
| Pimozide | – | – | 10 | 11 | 43 |
| Sulpiride | 40,000 | 80,000 | 10 | 20 | 1,000 |
| Raclopride | 10,000 | – | 2 | 4 | 1,500 |
| Clozapine | 140 | 250 | 70 | 300 | 9 |
| (+) UH232 | – | – | 40 | 10 | – |

[a]Dissociation constants expressed in nM. Values taken from references 26–28, 30, 34, 36, and 76.

## $D_2$ AND $D_3$ RECEPTORS AS COMMON TARGETS FOR ANTIPSYCHOTIC DRUGS

Before the advent of molecular biology, it had been generally assumed that neuroleptics derived their antipsychotic activity from the blockade of a single $D_2$ receptor.[16] Nevertheless, the recent discovery of several $D_2$-like receptor subtypes raises the possibility that the antipsychotic effects result in more discrete blockade of a particular subtype. We compared the binding data in the literature for several antipsychotic drugs used in clinical practice, at cloned DA receptors subtypes (TABLE 1). The low affinities at $D_1$-like receptors of several antipsychotic drugs, including the substituted benzamides sulpiride and raclopride and to a lesser extent haloperidol, indicate that blocking of these receptors is probably not achieved during treatment. In contrast, antipsychotic drugs have higher affinities at receptors of the $D_2$-like subfamily: $D_2$ and $D_3$ receptors appear to represent common targets for these drugs, with affinities in the nanomolar range for all compounds listed. This suggests that antipsychotic drugs produce their clinical effects primarily by blocking $D_2$ and $D_3$ receptors. In agreement, the study of brain of schizophrenic patients by positron emission tomography[17] indicates that antipsychotic drugs at clinically active dosages occupy the striatal $D_2$ receptor by 65–85%, a figure which is probably not very different for the $D_3$ receptor, given its similar pharmacological properties. In

addition, both $D_2$ and $D_3$ receptors are highly expressed in brain limbic structures,[18] where DA is involved in various aspects of behavior, mood, and cognition through a feedback with cortical activities. Disturbances at this level may participate in the etiology of schizophrenia. However, the $D_2$ receptor, unlike the $D_3$ receptor, is also highly expressed in dorsal striatum, a region implicated in the control of motor activity and in the pituitary, where DA controls prolactin release. This suggests that blockade of the $D_2$ receptor also produces the motor and endocrine adverse effects of antipsychotic drugs, a drawback of present antipsychotic medication that would not meet $D_3$ receptor blockers. Clinical assessment of putative antipsychotic compounds with $D_3$-preferring affinity, such as (+) UH232 (TABLE 1), will allow us to evaluate this hypothesis.

The $D_4$ receptor also recognizes antipsychotic drugs with high affinity, but with a much higher variability. Particularly, it seems unlikely that the antipsychotic properties of raclopride[19–21] are due to the blockade of the $D_4$ receptor, for which this compound has a very low affinity.[22] Nonetheless, clozapine, an atypical antipsychotic drug that is relatively free of the adverse effects of drug-induced parkinsonism and tardive dyskinesia, binds to the $D_4$ receptor with an affinity at least 10 times higher than to other DA receptor subtypes. However, it should be noted that antipsychotic drugs, such as clozapine, have additional serotonergic,[23] muscarinic,[24] and $\alpha$-1 adrenergic[25] properties, which may be responsible for peculiar pharmacological profiles and/or atypical properties. Hence, it has been suggested that atypical properties correlate with dopamine $D_2$/serotonin $5HT_2$ receptor pKi ratios.[23] This latter hypothesis is not inconsistent with the concept of a specific involvement of $D_2$ or $D_3$ receptors in antipsychotic drug effects, if serotonin or another transmitter interacting with extrapyramidal systems may prevent the negative consequences of dopamine receptor blockade on motor control. It seems likely, indeed, that an atypical antipsychotic profile may be achieved in more than one way.

## A FUNCTIONAL *IN VITRO* MODEL FOR $D_3$ RECEPTOR ACTIVATION

Heterologous cell expression systems allowed us to identify the second messenger pathways of DA receptor subtypes. The $D_1$[26–29] and $D_5$[30,31] receptors stimulate adenylyl cyclase, whereas $D_2$[5,6] and $D_4$[7] inhibit this enzyme activity. The $D_2$ receptor is functionally coupled to additional effector systems: it decreases $Ca^{2+}$ influx by activating $K^+$ channels and activates phospholipase C in some cells,[6] but it inhibits this enzyme in other systems.[32] $D_2$ receptors also activate arachidonic acid release, provided that phospholipase $A_2$ is stimulated by raised intracellular $Ca^{2+}$.[12,33] All these effects are mediated via GTP-binding proteins (G-proteins) of the $G_i/G_o$ group, which also regulate agonist binding. Binding at $D_2$ and $D_4$ receptors in membranes is generally described as occurring in two affinity states, the high-affinity state being converted into the low-affinity state by GTP.[5,34]

The evidence for such coupling of the $D_3$ receptor to G-proteins has long been lacking. In various transfected cells, including transfected fibroblasts such as Chinese hamster ovary cells (CHO), no[35] or little[8,36–38] GTP-induced shift in agonist affinity could be observed, as well as inconsistent inhibition of adenylyl cyclase[36,37] and weak activation of phospholipases.[12,38] The lack of indication of $D_3$ receptor coupling to conventional effector systems may seem paradoxical, given the sequence homology this receptor displays with the $D_2$ receptor in the third intracytoplasmic loop, a part of the receptor presumably implicated in coupling to G-proteins. One possible explanation is that the recipient cells used in previous studies may not be appropriate, either because the $D_3$ receptor is incorrectly processed or integrated in the

membrane or because the cells do not express the adequate G-protein or effector system.

In view of the above considerations, we sought an appropriate recipient cell by transfecting a neuroblastoma-glioma NG 108-15 hybrid cell line,[39] the neuronal origin of which may afford a repertoire of effectors and G-proteins more extended than in the CHO cell line. Accordingly, in contrast with this latter cell line, the transfected NG108-15 cell line expresses a $D_3$ receptor that exists in two affinity states, interconverting by GTP analogs. The relevance of this observation to the physiological function of the $D_3$ receptor is indicated by the similar effects found under identical experimental conditions in membranes of lobules 9 and 10 of rat cerebellum, where a pure population of constitutive $D_3$ receptors can be studied.[40] Previous observations (P. Sokoloff and C. Pilon, unpublished results) indicated that

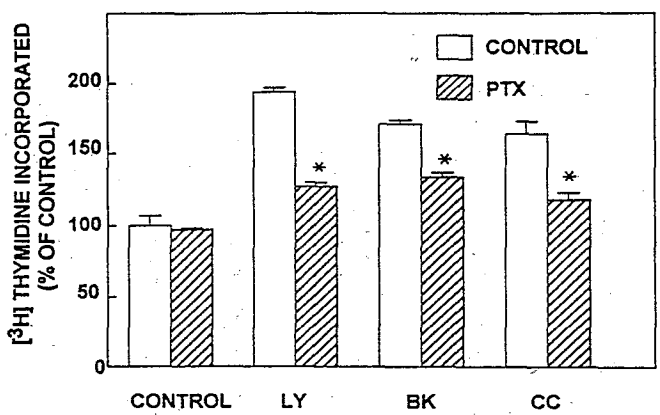


FIGURE 1. Effect of pertussis toxin (PTX) on stimulation of mitogenesis induced by quinpirole, bradykinin or carbamylcholine. Cells were pretreated for 24 h with pertussis toxin (200 ng/mL) stimulated by 0.1 μM quinpirole (LY), 1 μM bradykinin (BK) or 100 μM carbamylcholine (CC). Mitogenesis was evaluated by measuring [$^3$H]thymidine incorporation. Results are expressed as percent of radioactivity incorporated in unstimulated cells. Asterisk denotes a significant difference ($p < 0.002$) in treated cells (*hatched columns*) versus untreated cells (*open columns*) by the Student's *t* test.

the effects of guanylnucleotides on agonist binding at $D_3$ receptors expressed by transfected CHO cells were enhanced by co-transfecting an α-subunit cDNA of $G_o$. This suggested that a G-protein of this type, constitutively expressed in the NG 108-15,[39] but not in the CHO cell line,[41] naturally couples to the $D_3$ receptor. Accordingly, $D_3$ receptor stimulation in transfected NG 108-15 increases mitogenesis through a pertussis toxin-sensitive mechanism (FIG. 1). Previous studies have indicated that a wide variety of G-protein-coupled receptors are able to induce mitogenesis.[42] Several concomitant and interacting mechanisms probably contribute to mitogenesis, which complicates the identification of the initial second messenger signaling pathways involved. Potent mitogenic factors either increase phosphatidylinositol turnover (see FIG. 2 for effects of bradykinin) or inhibit forskolin-stimulated cyclic AMP formation (see FIG. 2 for effects of carbamylcholine). The $D_3$ receptor-

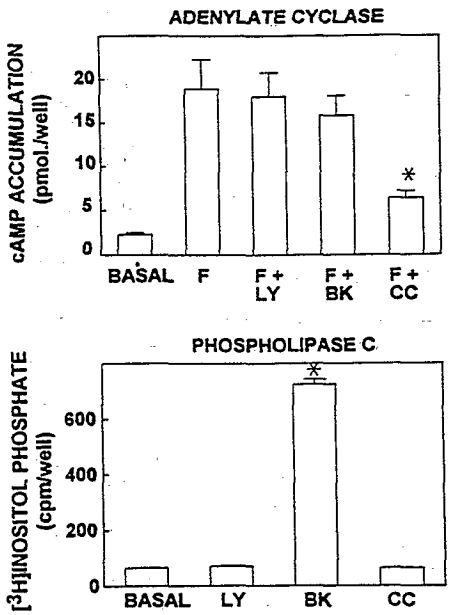


FIGURE 2. Effects of quinpirole, bradykinin or carbamylcholine on cyclic AMP (*top*) and inositol phosphate (*bottom*) accumulations. Cyclic AMP accumulation was measured in cells stimulated by 10 μM forskolin (F). LY, quinpirole, 0.1 μM; BK, bradykinin, 1 μM; CC, carbamylcholine, 100 μM. Asterisk denotes a significant difference ($p < 0.01$) versus forskolin alone (*top panel*) and ($p < 0.001$) versus basal level (*bottom panel*), by the Student's *t* test.

induced mitogenesis does not appear to result from increased production of phospholipase C-associated second messengers or inhibition of adenylyl cyclase (FIG. 2). Nevertheless, the increase of diacylglycerol, one of the phospholipase C products that activates protein kinases, may play a significant role in $D_3$ receptor-induced mitogenesis because a phorbol ester, which also activates protein kinases, potentiated the response.[39] Thus, the $D_3$ receptor produces mitogenesis by affecting a still unidentified pathway, through a pertussis toxin-sensitive mechanism.

This functional model now allows us to pharmacologically characterize a $D_3$ receptor-mediated response (TABLE 2). DA receptor agonists, some of which were previously classified as autoreceptor-selective agonists, appear as full $D_3$ receptor agonists with subnanomolar potencies; (+)UH 232, a partially selective $D_3$ receptor compound, which displays in animal models a peculiar behavioral pattern ascribed to selective autoreceptor blockade,[43] appears now as a reversible $D_3$ receptor antagonist.[39] Taken together, these pharmacological data support the previously suggested hypothesis[35,36] that some behavioral and biochemical actions of DA agonists in low dosages, which had been attributed to their selective interaction with DA autoreceptors, may actually involve the $D_3$ receptor. In addition, not only the $D_3$ receptor but also the $D_2$ receptor is able to enhance mitogenesis, which allowed us to compare the efficacies of various agonists at these two receptors in similar experimental conditions (TABLE 2). It is clear from this comparison that the $D_3$ receptor selectivity is much lower when biological activity is considered than in binding studies. For example, quinpirole and 7-hydroxy dipropyl aminotetralin (7OH-DPAT), which are 50 times more potent in binding studies, have only a 2–7 times higher potency in the functional models. This suggests that attempts to identify an *in vivo* $D_3$ receptor-mediated response by using only agonists that displayed $D_3$ receptor binding selectivity[44–46] should be considered with caution. It remains that the mitogenic

response in transfected NG108-15 is a suitable model for identifying selective $D_3$ receptor agonists, which may prove useful in the characterization of the $D_3$ receptor function in the brain.

## $D_3$ RECEPTOR-MEDIATED ACTIVATION OF THE c-*FOS* GENE IN TRANSFECTED CELLS

In transfected NG 108-15, the $D_3$ receptor stimulates the transcription of the proto-oncogene c-*fos*, as measured by the appearance of Fos immunoreactivity (FIG. 3). The c-*fos* gene, an immediate early gene rapidly and transiently expressed in a wide variety of cell types including neurons,[47,48] constitutes a marker for cell activity and is involved in cell differentiation-proliferation balance.[49–51] Activation of c-*fos* by a DA receptor has never been reported in transfected cells, even though c-*fos* is activated in brain upon administration of indirect DA agonists in normal animals[52,53] and of $D_1$ receptor agonists following 6-hydroxydopamine-induced denervation of the striatum.[54] Antagonists of $D_2$-like receptors, that is, $D_2$, $D_3$ or $D_4$ receptors, also activate c-*fos* transcription *in vivo*,[55–58] suggesting that a receptor of this type is tonically and negatively coupled to c-*fos* expression. $D_2$ receptor-mediated inhibition of both cAMP formation and of calcium channel activity presumably contributes to this negative coupling by counteracting the action of a calcium/cAMP-dependent responsive enhancer in the c-*fos* gene.[48] Such a process may be relevant for the therapeutic action of neuroleptics, because, although "typical" antipsychotic drugs induce c-*fos* in various parts of the striatal complex, including those involved in motor functions, the action of the "atypical" ones, such as clozapine and remoxipride, is

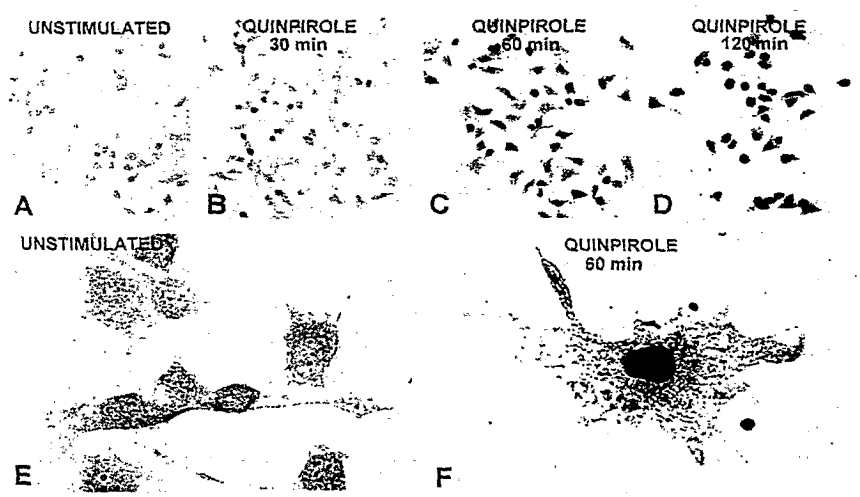


**FIGURE 3.** Induction of Fos-like immunoreactivity by quinpirole in transfected NG 108-15 cells. Monolayer cell preparations were incubated without (**A**) or with 0.1 μM quinpirole for 30 (**B**), 60 (**C**), or 120 min (**D**). The cells were stained with an anti-Fos polyclonal antibody. The intense coloration appearing after a 30-min stimulation by quinpirole is restricted to nucleus, which is shown in (**F**) at a higher magnification, whereas unstimulated cells display only a diffuse and weak coloration (**E**).

restricted to the limbic parts of the nucleus accumbens,[56–58] a region in which disturbances in DA neurotransmission have been implicated in schizophrenia.

## OPPOSING ROLES FOR $D_2$ AND $D_3$ RECEPTORS ON NEUROTENSIN EXPRESSION IN NUCLEUS ACCUMBENS

In contrast with the $D_2$ receptor, the $D_3$ receptor is almost absent from the dorsal striatum; like the $D_2$ receptor, however, it is well expressed in the ventral striatum, particularly the nucleus accumbens.[18,40,59] We have compared the distributions of $D_2$ and $D_3$ receptor mRNAs in accumbal subterritories, namely, shell and core, known to display distinct cytochemical features, connections, and therefore functions.[60–62] At the level of the island of Calleja major, where shell and core subterritories can be easily distinguished, $D_3$ receptor mRNA was almost exclusively expressed in the shell, whereas the $D_2$ receptor mRNA was expressed in the core as well as in restricted parts of the shell (FIG. 4). As in other brain areas,[18] however, no overlap is found between $D_2$ and $D_3$ receptor mRNA distributions in the shell. The $D_3$ receptor mRNA is expressed in the ventromedial area of the shell (ShV), which also expresses neurotensin (NT) but not $D_2$ receptor mRNA, whereas the reverse situation exists in a more dorsal shell area called the "cone" (ShC in FIG. 4).

Since the distribution of $D_3$ receptor and NT/neuromedin N mRNAs within the ShV seemed to overlap, a possible colocalization of these markers was assessed in thin successive sections (FIG. 4D and E). Both mRNAs are coexpressed in a subpopulation of medium-sized neurons representing 42% of the NT neurons in the analyzed area. In comparison, $D_3$ receptor mRNA is expressed by 43% of the total cellular population of the same area. Because these values are inherently minimized, it can be safely concluded that a major proportion of NT neurons of this area of the shell, known to heavily project to the ventromedial pallidum, express the $D_3$ rather than the $D_2$ receptor.

In the absence of highly selective $D_3$ receptor ligands, the role of dopamine innervation on these neurons was assessed by simultaneous blockade of $D_2$ and $D_3$ receptors using haloperidol or sulpiride, two antipsychotic drugs with a $D_2$-like selectivity (FIG. 5), in rather high dosage. In agreement with previous studies[13–16,63] these agents induced a marked activation of NT gene expression in $D_2$-receptor rich areas, for example, the dorsal striatum (not shown) or shell cone of accumbens (FIG. 5). In contrast, both agents induced opposite changes in the ShV, an area selectively expressing $D_3$ receptors; therein the preexisting NT mRNA signal is markedly decreased. Thus, in subterritories of the shell, the $D_3$ receptor may exert a tonic stimulation of NT expression, an effect opposite to that exerted in other striatal divisions by the $D_2$ receptor. Nevertheless, the increase in striatal NT expression triggered by antipsychotic drugs may be the indirect result of an increase in DA neuron firing, mediated by $D_2$ autoreceptor blockade, which results in a higher availability of DA at $D_1$ receptors.[64] However, SCH 23390, a selective dopamine $D_1$-like receptor antagonist, do not induce any change in NT mRNA in any region of the nucleus accumbens (FIG. 4C), supporting the involvement of a $D_2$-like receptor in the regulation of NT expression in this region.

A $D_3$ receptor-mediated activation of NT gene transcription is consistent with the detection of spontaneously expressed transcripts in $D_3$ receptor-rich accumbal areas. In addition, it is also consistent with the observations that, in transfected NG-108-15 cells, the $D_3$ receptor promotes activation of the c-*fos* gene, whose product is known to activate transcription of the NT gene via binding to its AP1 site.[65]

## REGULATION OF THE $D_3$ RECEPTOR AFTER INTERRUPTION OF DOPAMINERGIC TRANSMISSION

Prolonged interruption of DA neurotransmission in animals by chronic treatment with neuroleptics or by lesions of dopaminergic neurons results in behavioral

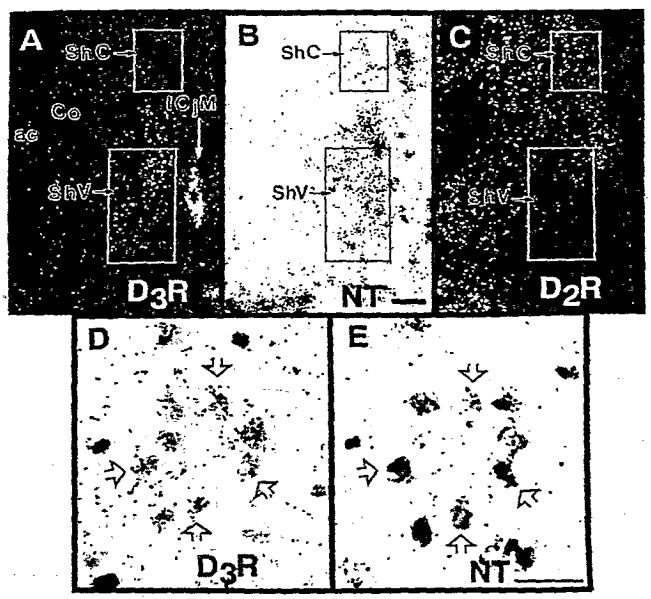


FIGURE 4. Expression patterns of $D_2$ and $D_3$ receptor mRNAs in the nucleus accumbens and comparison with neurotensin (NT) mRNA in control animals. Frontal sections performed at 1.2 mm to bregma (Paxinos and Watson, 1982) were hybridized with [$^{35}$S]labeled cRNA probes for $D_3$ receptor (**A**), NT (**B**) or $D_2$ receptor (**C**). Note that in the ventromedial part of the shell subdivision of the nucleus accumbens (area surrounded by a rectangle and subsequently referred to as ShV) the distribution of $D_3$ receptor mRNA matched the expression pattern of NT mRNA. In **A** and **C**, the microphotographs were obtained under darkfield illumination. On brightfield microphotographs of 3-μm sections hybridized with the $D_3$ receptor cRNA probe (**D**) and corresponding adjacent section hybridized with the NT cRNA probe (**E**) at the level of the ventromedial part of the shell, four of nine neurons present on both sections showed hybridization signals with both probes and are indicated by arrows. Ac, anterior commissura; Co, core subdivision of the nucleus accumbens; ICj M, island of Calleja Major; ShC, cone part of the shell subdivision; ShV, ventromedial part of the shell. Bars = 250 μm (**A–C**), 20 μm (**D, E**).

supersensitivity to DA agonists and increased number of receptors.[66] Elevated $D_2$ receptor mRNA[67–70] after chronic neuroleptic treatment is direct evidence that enhanced responsiveness via increase in DA receptor number results from increased rate of synthesis through activation of gene transcription. It is generally recognized, however, that no tolerance to the antipsychotic activity of neuroleptics develops in

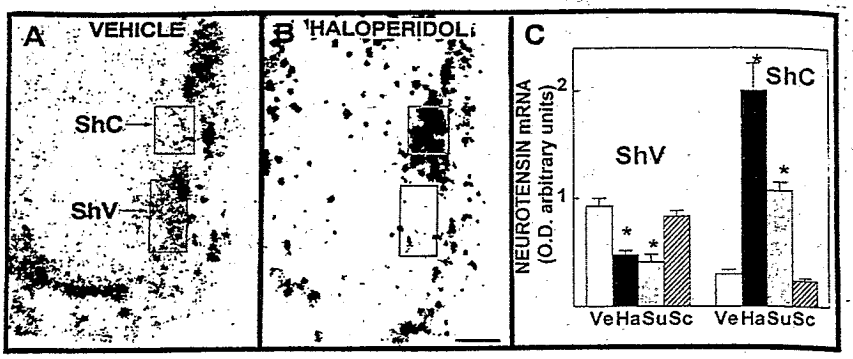


FIGURE 5. Effects of dopamine receptor antagonists on expression of NT mRNA. Frontal sections taken from a vehicle (A) or a haloperidol-treated animal (B) were hybridized with a neurotensin (NT) cRNA probe. Bar = 500 μm. In C, autoradiograms obtained as in A and B from 6–8 sections from groups of 3–5 rats receiving vehicle (Ve), haloperidol (Ha), sulpiride (Su) or SCH 23390 (Sc) were analyzed with an image analyzer (Biocom 2000, Les Ulis, France) in the ShV and the ShC subdivisions as delineated in A. *$p < 0.005$ as compared to vehicle-treated rats by the Mann-Whitney U test.

schizophrenic subjects,[71] whereas tolerance to the motor side effects is progressively setting in the course of the treatment. We compared the effects of dopaminergic neuron ablation and of a chronic haloperidol treatment on levels of $D_2$ and $D_3$ receptor binding and mRNA in the nucleus accumbens. After a two-week treatment, haloperidol enhanced both $D_2$ receptor mRNA and $D_2$ receptor binding in the nucleus accumbens. In contrast, neither $D_3$ receptor binding nor mRNA changed, suggesting that no up-regulation develops at this receptor. In addition, no tolerance to the effect of haloperidol on the activation of NT expression was seen (TABLE 3). This observation might be regarded as further support to the idea that the $D_3$ receptor is an important target for antipsychotic drugs. In contrast with their motor

TABLE 2. Comparison of Potencies of Dopamine and Agonists at $D_2$ and $D_3$ Receptors in Binding and Functional Studies

| | Receptor Binding ($K_i$ values, nM) | | | Stimulation of [$^3$H]Thymidine Incorporation ($EC_{50}$ values, nM) | | |
|---|---|---|---|---|---|---|
| Agonist | $D_2$ Receptor | $D_3$ Receptor | $K_i(D_2)/K_i(D_3)$ | $D_2$ Receptor | $D_3$ Receptor | $EC_{50}(D_2)/EC_{50}(D_3)$ |
| Dopamine | 544 | 23 | 24 | 20 | 1.4 | 15 |
| Apomorphine | 63 | 73 | 0.87 | 2.3 | 2.2 | 1.1 |
| Quinpirole | 1,400 | 39 | 36 | 2.8 | 0.86 | 3.3 |
| (+)7OH-DPAT | 103 | 2.1 | 49 | 2.7 | 0.39 | 7.0 |

NOTE: Data for binding at $D_2$ and $D_3$ receptors were taken from Sokoloff *et al.*[36] $EC_{50}$ values for $D_2$ and $D_3$ receptor-mediated stimulation of mitogenesis, evaluated by measuring the incorporation of [$^3$H]thymidine, were calculated from concentration-response curves obtained using Chinese hamster ovary cells transfected with the rat dopamine $D_2$ receptor cDNA and with NG 108-15 cells transfected with the human $D_3$ receptor cDNA, respectively.

side effects which progressively diminish during long-term treatments, their therapeutic effects do not show any impairment.

Interestingly, ablation of dopamine neurons by 6-hydroxydopamine injection (TABLE 3) or medial forebrain lesions (not shown) results in a dramatic but paradoxical decrease in $D_3$ receptor expression in the nucleus accumbens. This effect is not reproduced by blockade of $D_1$-like, $D_2$-like, and cholecystokinin receptors, suggesting that a messenger molecule released from catecholamine neurons—but distinct from dopamine or its co-transmitter[72] cholecystokinin—is necessary to maintain the expression of the $D_3$ receptor in the accumbens. This not only confirms that $D_2$ and $D_3$ receptors are opposites in terms of their roles and regulation, but also suggests that identification of this putative messenger molecule will constitute a heuristic research area in the pathophysiology of schizophrenia.

TABLE 3. Effects of Interruptions of Dopamine Neurotransmission on the Expression of $D_2$ and $D_3$ Receptors and Neurotensin in the Nucleus Accumbens

| | $D_2$ Receptor | | $D_3$ Receptor | |
|---|---|---|---|---|
| Treatment | mRNA | Binding | mRNA | Binding |
| 6-OHDA | $+59 \pm 25^a$ | $+42 \pm 6^a$ | $-52 \pm 10^a$ | $-54 \pm 12^b$ |
| Haloperidol | $+61 \pm 12^b$ | $+66 \pm 6^b$ | $-3 \pm 20$ ns | $+7 \pm 4$ ns |

NOTE: In the lesion study, animals received a unilateral injection of 6-hydroxydopamine (6-OHDA) and were killed 3 weeks later. Treatment with haloperidol consisted of either twice daily injections of haloperidol (20 mg/kg) for 2 weeks when $D_2$ and $D_3$ receptor mRNA and binding were measured or a single injection when neurotensin mRNA was measured. Binding at $D_2$ and $D_3$ receptors was measured using [$^{125}$I]iodosulpride[74] and [$^3$H]7OH-DPAT,[42] respectively. mRNA levels were measured by quantitative PCR with internal standards.[75] Neurotensin mRNA was measured by analyzing with an image analyzer the autoradiograms obtained in *in situ* hybridization experiments. Data are percent changes over mean values obtained in contralateral side or in vehicle-treated animals.

$^a p < 0.05$ and $^b p < 0.01$ by the Mann-Whitney test; ns, not significant.

## CONCLUSIONS

Neuroleptics share the common characteristic of being recognized by the DA $D_2$ and $D_3$ receptors, the blockade of which may, therefore, represent the primary mechanism for antipsychotic drug action. In addition, both $D_2$ and $D_3$ receptors are well expressed in brain limbic structures, such as the shell part of the nucleus accumbens, where DA neurotransmission is involved in various aspects of behavior, mood, and cognition through a feedback loop controlling cortical activities. Disturbances at this level may participate in the etiology of schizophrenia.

From functional studies, it appears, however, that $D_2$ and $D_3$ receptors act in opposite directions in the nucleus accumbens. The $D_2$ receptor exerts a tonic inhibition on c-*fos* and neurotensin gene transcriptions, whereas the $D_3$ receptor tonically stimulates NT expression and promotes c-*fos* expression, at least in transfected cells. That DA exerts opposite effects via $D_2$ and $D_3$ receptors is consistent with data of behavioral studies in rodents: agonists of $D_2$-like receptors (i.e., $D_2$, $D_3$ or $D_4$) induce contrasting locomotor manifestations when applied in different accumbal subterritories.[73] In addition, the partially selective $D_3$-antagonists AJ 76 and UH 232 trigger, in low dosage, paradoxical behavioral activations, originally

attributed to autoreceptor blockade because they resemble those of $D_2$ agonists; at somewhat higher dosage, however, these experimental drugs induce the behavioral disruptions characteristic of neuroleptics, that is, preferential $D_2$ antagonists.[43]

The discovery of the dual effects of $D_2$ and $D_3$ receptors on accumbal neurons may be relevant to the treatment of schizophrenia. Neuroleptics are more effective in alleviating positive symptoms such as hallucinations and delusions, than negative symptoms such as impoverished thought and affect. The occurrence of these distinct, even opposite, manifestations, sometimes in the same patient, suggests that the antipsychotic drugs do not normalize a single overactive dopaminergic pathway. The $D_2$ preference of the drugs presently available might be responsible for the greater efficiency of these drugs against positive rather than negative symptoms, and, in addition, with the drug-induced deficient symptoms in patients with schizophrenia. The forthcoming introduction in clinics of first $D_3$ receptor selective antagonists may contribute to the improvement in the treatment of schizophrenia.

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